GENOTYPE DEPENDENT HORMONAL REGULATION OF GROWTH AND IAA-OXIDASE ACTIVITY OF NORMAL AND GENETICALLY TUMOROUS TOBACCO TISSUE CULTURES

by

E. I. KOVÁCS

Biological Exp. Station, Eötvös L. University, H–2131 Göd

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Morphogenesis and endogenous indoleacetic acid (IAA) level are controlled by the indoleacetic acid oxidase enzyme system (Siegel–Galston 1967, Galston – Davies 1969, Elkinawy – Raa 1973, Kovács 1977, 1978). Although there are known instances in which the exogenous addition of hormones specifically influences the activity of some enzymes (Chrispeels – Varner 1968, Varner – Ho 1977, Gaspar et al. 1982) little is known about hormonal regulation of IAA oxidase enzyme in tissue cultures of different genotype. For this reason regulation of growth, IAA oxidase activity and the interaction between genotype and hormones were studied.

Materials and methods

Tissue cultures of *Nicotiana glauca* (normal) and the tumor forming F_1 hybrid of *N. glauca* x *N. langsdorffii* were grown under the experimental conditions described before (Kovács 1967, 1971). In studies of IAA (indoleacetic acid) and 2,4-D (2,4-dichlorophenoxyacetic acid) the basal medium (Kovács 1971) contained 0,2 mg/l kinetin (KIN) while in the kinetin experiments the basal medium contained 2,0 mg/l IAA. Fresh weight and IAA oxidase activity of the 3-week-old cultures were determined.

IAA oxidase activity of dialysed aceton-powder extracts of the cultures was measured: Reaction mixture : 100 mM NaH_2PO_4, 0,1 mM $MnCl_2$, 0,1 mM 2,4-dichlorophenol, 0,2 mM IAA and enzyme extract in 2,0 ml volume. The reaction mixture was shaken for 10 min at 25 °C then the reaction was stopped by addition of Gordon and Weber's reagent (1951) and 40 min later the optical density of the solution was measured at 530 nm. One enzyme unit (EU) is 1 μmole of destroyed IAA per 10 min at 25 °C. Enzyme activity is expressed as EU per g fresh weight. The data were subjected to statistical analysis (Sváb 1967).

Results

Fresh weight growth of normal tissues is enhanced by increasing concentrations of IAA but the same treatments are ineffective or inhibitory (10^{-4} M IAA) for growth of tumorous cultures. (Fig. 1). 10^{-7} M 2,4-D increases growth of normal cultures, only. The higher concentrations of 2,4-D are inhibitory for growth of both normal and tumorous tissues, too (Fig. 1). The same is true for the KIN treatments. Above 10^{-6} M concentration effect of KIN is rather inhibitory for growth of both normal and tumorous cultures (Fig. 1). The Fig. 1 and Table 1 clearly show that effect of the hor-

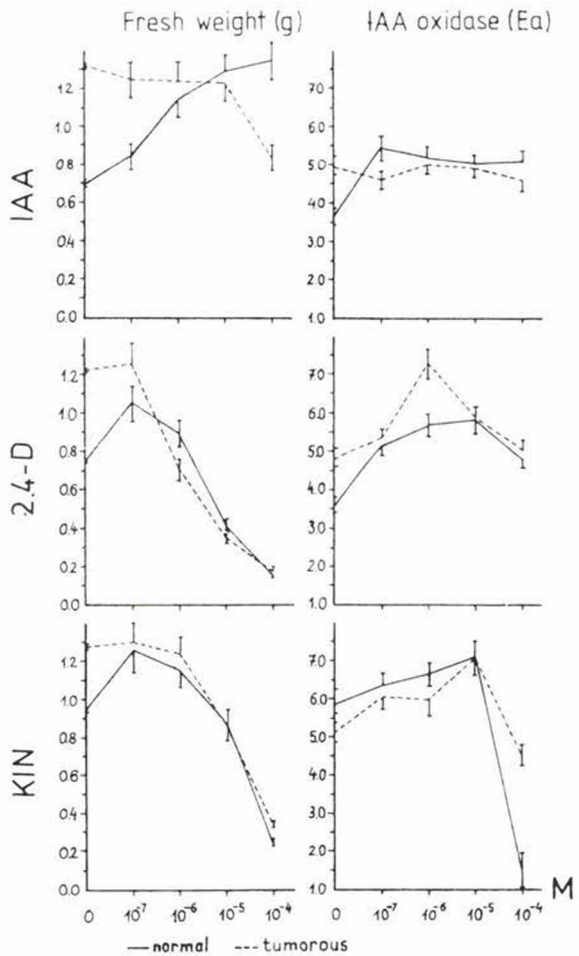

Fig. 1. Effect of IAA, 2,4 – D, and KIN on growth and IAA oxidase enzyme activity (Ea) of normal and tumorous tobacco tissue cultures. (Ordinates: g fr. wt. and Ea in EU per g fr. wt.; abscisse: hormone concentrations in M. The vertical bars are standard errors.)

mones used depends on genotype of the tissues (genotype-hormone interaction).

IAA treatments stimulate IAA oxidase enzyme activity of normal cultures compared to the untreated ones, however, above the maximum IAA effect at 10^{-7} M the higher concentrations rather reduce the IAA oxidase level of tissues. These IAA treatments are rather ineffective to the tumorous cultures (Fig. 1).

2,4-D increases activity of the enzyme of both normal and tumorous cultures in a range of $10^{-7} - 10^{-5}$ M concentrations (10^{-4} M has a moderate stimulatory effect). The dose curve has a maximum at 10^{-6} M for tumorous cultures and at 10^{-5} M for the normal ones. The stimulatory effect of 2,4-D on IAA oxidase activity is higher in tumorous tissues than in the normal ones (Fig. 1).

KIN stimulates the IAA oxidase activity of normal tissues in higher degree than that of tumorous cultures (between $10^{-7} - 10^{-5}$ M). At 10^{-4} M concentration KIN has a strong inhibitory effect in both of the cultures (Fig. 1).

Under optimum conditions there is a correlation between growth of the cultures and their IAA oxidase level (Fig. 1). IAA induces IAA oxidase activity only in the cultures of normal genotype while 2,4-D and KIN can increase the enzyme activity in the cultures of both genotypes (Fig. 1).

Table I

Analysis of variance of hormonal effects on growth and IAA oxidase activity in tobacco tissue cultures

Item	d. f.	Growth		IAA oxidase	
		MQ	P%	MQ	P%
IAA treatment	9	0.2234	<0.1	0.9736	<0.1
effect of IAA	4	0.0895	0.1	0.8223	<1.0
effect of genotype	1	0.1245	<1.0	0.0462	>10.0
genotype-hormone interaction	4	0.3820	<0.1	1.3567	<0.1
Error	27	0.0140	–	0.1525	–
2,4-D treatment	9	0.6802	<0.1	3.6240	<1.0
effect of 2,4-D	4	0.3202	<0.1	6.1970	<1.0
effect of genotype	1	0.0799	<0.1	4.2200	<1.0
genotype-hormone interaction	4	1.1907	<0.1	0.9020	<1.0
Error	27	0.0023	–	0.1610	–
KIN treatment	9	0.5905	<0.1	11.2800	<0.1
effect of KIN	4	1.2643	<0.1	20.3100	<0.1
effect of genotype	1	0.1128	<0.1	1.3200	<1.0
genotype-hormone interaction	4	0.0361	<0.1	4.7500	<0.1
Error	27	0.0004	–	0.1600	–

Note: in the analysis means of separate experiments of the experimental series were used (S v á b 1967); the hormone treatment and the error of the complete analysis are published, only.

That is the hormonal effect depends on genotype of the cultures (genotype-hormon interaction; Tabl. 1). A highly significant genotype-hormone interaction is shown by variance analysis of the growth and the IAA oxidase data of four experiments (Tabl. 1). The genotype-IAA interaction is the most powerful while genotype-2,4-D interaction is moderate. In spite of these facts the hormonal treatments are more efficient on growth and IAA oxidase level of cultures than effect of genotypes (Tabl. 1).

Discussion

The experimental results demonstrate that growth regulation of tissue cultures involves the IAA oxidase enzyme system depending on growth conditions of cultures. In restrictive conditions growth regulating role of IAA oxidase is limited (e.g. absence of essential factors, hormones; toxic effects – toxic 2,4-D and KIN concentrations –, etc.). In permissive conditions (optimum growth conditions) IAA oxidase system can regulate growth of cultures (Fig. 1).

Permissive or restrictive effects of culture conditions on tissues grown in vitro depend on their genotype. This fact is suggested by the significant genotype-hormone interaction found in the hormonal influence of growth and IAA oxidase level of cultures (Fig. 1 and Tabl. 1). The analysis of variance clearly shows that the genotype itself is less effective on growth, morphogenesis and IAA oxidase synthesis than either hormones or both hormones and genotype together. The genotype-hormone interaction is synergistic (Tabl. 1). These results and the absolute hormone requirement (auxin and cytokinin together) of IAA oxidase synthesis suggest that regulation of genes coding the IAA oxidase system may be under positive control. The positive control of some eukaryotic genes is supported by other data (K o v á c s 1978, B l i s s 1981). The genotype-hormone interaction, itself, means a direct influence of hormones on regulation of gene expression.

On the basis of the experiments presented biochemical basis of the genotype-hormone interaction is explained by differential destruction of endogenous IAA by the IAA oxidase systems of the cultures produced differentially by normal and tumorous genotype. The exogenous IAA is destroyed by the tumorous cultures in higher degree than by the normal ones and their low endogenous IAA level is not suitable for increase of the enzyme synthesis. This explains the relative ineffectiveness of the added IAA on tumorous cultures. 2,4-D and KIN cannot be destroyed by the IAA oxidase, thus, they are more effective for each culture (Fig. 1).

The increased IAA destruction of tumorous cultures brings about a relatively high cytokinin level maintaining the high IAA oxidase level (feedback control). This statement is confirmed by the data presented in Fig. 1 and it is also supported by the facts that KIN treatmet increases activity of IAA and indoleacrylic acid oxidizing enzyme systems of lentil roots (G a s p a r – X h a u f f l a i r e 1967, D a r i m o n t et al. 1971) and of some IAA oxidase isoenzymes of tobacco tissue cultures (L e e

1972, 1974). The relatively high cytokinin to auxin ratio can cause increased bud and shoot formation as well as tumor formation in tissues of the hybrid tobacco plants (Kovács 1977). These are in keeping with the concept of chemical regulation of organogenesis (Skoog – Miller 1957).

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Summary

Effects of IAA, 2,4-D and kinetin on growth and IAA oxidase activity of tobacco tissue cultures of normal and tumorous genotype were studied. IAA oxidase regulates growth of cultures in permissive conditions, only. IAA, 2,4-D and kinetin increase the IAA oxidase activity at lower concentrations. The hormonal effect on growth and on enzyme activity depends on the genotype of tissues. Variance analysis of the growth and IAA oxidase experiments showed a significant genotype-hormone interaction. The differential IAA destruction of tissues of different genotype is a step in the biochemical basis of this interaction. Regulation of growth, organization and IAA oxidase activity of normal and tumorous cultures involves this genotype-hormone interaction.

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